Metal Chelates to Prevent or Clear the Deposits of Amyloid β-peptide(1-40) induced by Zinc(II) Chloride

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We have observed that some metal compounds can dissociate the aggregated proteins in solution, and can prevent or clear the deposits of amyloid β-peptide(1-40) induced by zinc(II) ion.

It has been pointed out that the cause of Alzheimer's disease (AD) is closely related to the aggregation of a normal protein, β-amyloid (Aβ), within the neocortex, and evidence has been gathered to suggest that Aβ precipitation and toxicity in AD are cused by abnormal interactions with neocortical metal ions, especially Zn, Cu, and Fe.[1,2] Zn^{2+} appears to be the major neurochemical factor responsible for aggregating Aβ, and Cu and Fe are found at markedly high levels in cerebral Aβ deposits, but the latter mechanism remains unanswered at present.[1,2] On the basis of the amyloid cascade model, the main therapeutic strategies have aimed either to prevent the production of Aβ, or to remove all forms of Aβ.

On contrast to above discussion, recent reports suggest that the toxicity of Aβ and other amyloidogenic proteins lies not in the insoluble fibrils that accumulate but rather in the soluble oligomeric intermediates.[3,4] The oligomers represent protein micelles, because oligomer formation displays a critical concentration dependence, and their formation is correleated with the appearance of a hydrophobic environment. These results indicate that the soluble oligomers may be more important pathologically than are the fibrillar deposits, but methods to detect the oligomers in solution are greatly limited at present. Very recently Lorenzi et al. have reported that capillary electrophoresis method (CE)[5] is useful to monitor the steps of β-amyloid nucleation in solution.[6] Here we will report that CE method is very suitable to investigate the dynamic behavior of proteins in solution including amyloid β-peptide(1-40) and superoxide dismutase (SOD),[7] and point out that some metal compounds can prevent or clear the Aβ deposits in the solution.

It is generally recognized that CE data are informative to investigate the aggregation of proteins in solution;[6,8,9] i.e., CE peak of the oligomer is generally sharper and higher than that of the corresponding monomer; for example, the CE profile of SOD (bovine) is sharper and stronger compared with that of transferrin of the same concentration (see Figure 1), and this is due to that SOD exists as a dimer in the solution.[10,11] When manganese(II) complex with (dpgt) (for the structures of the ligands, see Figure 2) was added to the SOD solution, the peak height at 5 min. was immediately lowered, but it gradually has become to be the height of the original solution (see Figure 3, B and C), but such behavior was not observed for the Mn(II) complex with (dpal) (see Figure 2). These fatcs clearly indicate that Mn(dpgt) complex induces the dissociation of the dimeric structure of SOD, and after the first shock, SOD has become to be the original dimeric structure.

Figure 1. CE profiles of the proteins. A: SOD(2 mg/1 mL solution); B, transferrin(2 mg/1 mL).

Figure 2. Structures of the ligands.

We have observed that Cu(bdpg-His)[12] (for the structures of the ligands, see Figure 2) induces the reduction (ca. 70%) of the peak height of SOD in the CE, suggesting that this copper(II) complex dissociates the dimeric structure of SOD. The decrease (ca. 60%) of intensity of the peak corresponding to the oligomeric species of Aβ(1-40) (peak at 5.5 min in Figure 4) is induced by the addition of Cu(bdpg-His) within 1 h as illustrated in Figure 4, implying that the degradation of oligomeric structure of the protein. This seems to be consistent with the fact that the addition of Cu(bdpg-His) greatly depresses the deposits of Aβ(1-40) induced by zinc(II) chloride,[13] as shown in Figure 5.

As Cu(bdpg-His) complex has an imidazole group as an anchor group,[14] which may interact with a Zn^{2+} ion, preventing the aggregation of Aβ protein in solution. These suggest that a metal compound which dissociate the dimeric protein with beta-sheet conformation may clear the amyloid deposits in solution. In fact,

Figure 3. Time dependence of peak height at 5 min. of SOD (1 mg/1 mL solution) by the addition of Mn(dpgt)Cl$_2$ (0.2 mg/1 mL solution). A: measured immediately after addition of Mn(II) chelate; B: after 30 min. C: after 60 min.
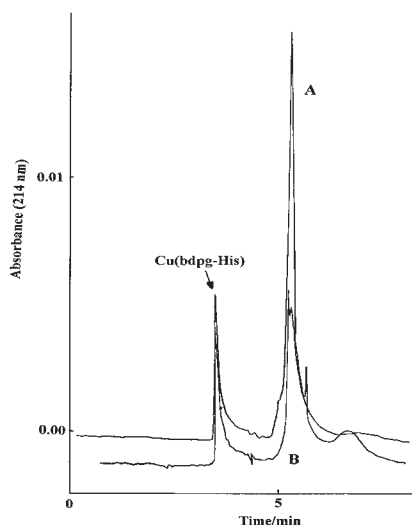

Figure 4. Time Dependence of CE peak at ca. 5.5 min (corresponds to aggregated Aβ(1-40)) of solution containing Aβ(1-40) (0.25 mg/0.5 mL) and Cu(bdpg-His)ClPF$_6$(2 mg/1 mL). A: measured immediately after solution was prepared. B: after 60 min.

Fe$_2$(HPTP)Cl$_4{}^+$,[15] which exhibits high activity to degrade the dimeric structure of SOD, can clear the amyloid deposits induced by Zn^{2+} (not shown). These may give important information to develop the new therapies for AD.
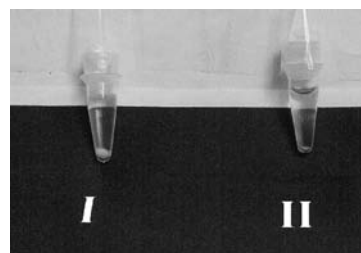
Figure 5. Deposition of Aβ(1-40) by zinc(II) chloride. I: Aβ(1-40)(0.5 mg/2 mL) and Zn(II)Cl$_2$(100 mM). II: Cu(bdpg-His)ClPF$_6$(1 mg/1 mL) was added to solution A.

References and Notes

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12 Cu(bdpg-His)ClPF$_6$ was prepapred from Cu(dpal)Cl and histidine methyl ester by the use of WSCD. Crystal structure determination revealed that this complex exists as a dimeric one in solid state; the copper(II) ion is coordinated by three nitrogen atoms of two pyridine molecules and tertiary aliphatic amine, and chloride ion, and the imidazole group of the histidine moiety is coordinating to the another copper ion. Crystal data of Cu(bdpg-His)ClPF$_6$·H$_2$O (CCDC 200670): monoclinic, space group $P2_1$(#4), $a = 13.8588(9)$, $b = 21.585(2)$, $c = 8.8880(5)$ A, $\beta = 89.948(4)$ degree, V = 3028.3(3) A^3, $R = 0.078$ for 6609 reflections with $I > 2\sigma(I)$.
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